$\tt CGCAGAAACTCGCGGCGGCAGTCTTGTTCCGTACATAATCTTGGTCTGCAATAATGGGAGAAGCTCTGAAGTACAGTATCATGGAC$ MGEALKYSIMD TCAGTAAGATCGGTAGTTTTCAAAGAATCCGAAAATCTAGAAGGTTCTTGCACTAAAATCGAGGGCTACGACTTCAATAAAGGCCGT SVRSVVFKESENLEGSCTKIEGYDFNKGV TAACTATGCTGAGCTGATCAAGTCCATGGTTTCCACTGGTTTCCAAGCATCTAATCTTGGTGACGCCATTGCAATTGTTAATCAAA N Y A E L I K S M V S T G F Q A S N L G D A I A I V N Q TGCTAGATTGGAGGCTTTCACATGAGCTGCCCACGGAGGATTGCAGTGAAGAAGAAGAAGATGTTGCATACAGAGAGTCGGTAACC M L D W R L S H E L P T E D C S E E E R D V A Y R E S V T TGCAAAATCTTCTTGGGGTTCACTTCAAACCTTGTTTCTTCTTGTTTAGAGACACTGTCCGCTACCTTGTTCAGCACCGGATGGT C K I F L G F T S N L V S S G V R D T V R Y L V Q H R M V TGATGTTGTGGTTACTACAGCTGGTGGTATTGAAGAGGGTCTCATAAAGTGCCTCGCACCAACCTACAAGGGGGGACTTCTCTTTAC D V V V T T A G G I E E D L I K C L A P T Y K G D F S L CTGGAGCTTCTCTACGATCGAAAGGATTGAACCGTATTGGTAACTTATTGGTTCCTAATGACAACTACTGCAAATTTGAGAATTCG P G A S L R S K G L N R I G N L L V P N D N Y C K F E N W ATCATCCCAGTTTTTGACCAAATGTATGAGGAGCAGATTAATGAGAAGGTTCTATGGACACCATCTAAAGTCATTGCTCGTCTGGG I I P V F D Q M Y E E Q I N E K V L W T P S K V I A R L G K E I N D E T S Y L Y W A Y K N R I P V F C P G L T D G CACTTGGTGACATGCTATACTTCCATTCTTTCAAAAAGGGTGATCCAGATAATCCAGATCTTAATCCTGGTCTAGTCATAGACATT S L G D M L Y F H S F K K G D P D N P D L N P G L V I D I GTAGGAGATATTAGGGCCATGAATGGTGAAGCTGTCCATGCTGGTTTGAGGAAGACAGGAATGATTATACTGGGTGGAGGGCTGCC V G D I R A M N G E A V H A G L R K T G M I I L G G G L P TAAGCACCATGTTTGCAATGCCAATATGATGCGCAATGGTGCAGATTTTGCCGTCTTCATTAACACCGCACAAGAGTTTGATGGTA K H H V C N A N M M R N G A D F A V F I N T A Q E F D G GTGACTCTGGTGCCCGTCCTGATGAAGCTGTATCATGGGGAAAGATACGTGGTGCTGCCAAGACTGTGAAGGTGCATTGTGATGCA S D S G A R P D E A V S W G K I R G G A K T V K V H C D A ACCATTGCATTTCCCATATTAGTAGCTGAGACATTTGCAGCTAAGAGTAAGGAATTCTCCCAGATAAGGTGCCAAGTTTGAACATT TIAFPILVAETFAAKSKEFSQIRCQV GAGGAAGCTGTCCTTCCGACCACACATATGAATTGCTAGCTTTTGAAGCCAACTTGCTAGTGTGCAGCACCATTTATTCTGCAAAA CCATGTTATTTAGTTCTCTTCCTCTTCGAAAGTGAAGAGCTTAGATGTTCATAGGTTTTGAATTATGTTGGAGGTTGGTGATAACT GACTAGTCCTCTTACCATATAGATAATGTTATCCTTGTACTATGAGATTTTGGGTGTGTTTGATACCAAGGAAAAATGTTTATTTGG

### Arabidopsis DeoxyHypusine Synthase (DHS) Predicted Sequence

Figure 2A sigtD=5 = NT GAACTCCCAAAACCCTCTACTACTACACTTTCAGATCCAAGGAAATCAATTTTGTCATTCGAGCAACATGG 

EDDRVFSSVHSTVFKESESLEGK GATAAAATCGAAGGATACGATTTCAATCAAGGAGTAGATTACCCAAAGCTTATGCGATCCATGCTCACCAC K I E G Y D F N Q G V D Y P K L M R G F Q A S N L G E A I D V V N Q M

CAAAAATAAAAATTCCTTCTTTTTGTTTTCCTTTGTTTTGGGTGAATTAGTAATGACAAAGAGTTTGAATT E F

TGTATTGAAGCTAGATTGGAGACTGGCTGATGAAACTACAGTAGCTGAAGACTGTAGTGAAGAGGAGAAGA V L K L D W R L A D E T T V A E D C S ATCCATCGTTTAGAGAGTCTGTCAAGTGTAAAATCTTTCTAGGTTTCACTTCAAATCTTGTTTCATCTGGT SFRESVKCKIFLGFTSNL V R D T I R Y L V QHH

TTATAGATGTTAAAATTTTCGAGCTTTAGTTTTGATTTCAATGGTTTTTCTGCAGGTTGATGTTATAGTCA

CGACAACTGGTGGTGTTGAGGAAGATCTCATAAAATGCCTTGCACCTACATTTAAAGGTGATTTCTCTCTA TTTGGVEEDLIKCLAP CCTGGAGCTTATTTAAGGTCAAAGGGATTGAACCGAATTGGGAATTTGCTGGTTCCTAATGATAACTACTG PGAYLRSKGLNRIGNLLVPNDNY CAAGTTTGAGGATTGGATCATTCCCATCTTTGACGAGATGTTGAAGGAACAGAAGAAGAGGTATTGCTTT K F E D W I I P I F D E M L K E Q K

TCTTGCATCATTGACTTCGTTGGTGAATCCTTCTTTCTCTGGTTTTTCCTTGTAGAATGTGTTGTGGACTC

CTTCTAAACTGTTAGCACGGCTGGGAAAAGAAATCAACAATGAGAGTTCATACCTTTATTGGGCATACAAG P S K L L A R L G K E I N N E S SYLY **GT**ATCCAAAATTTTAACCTTTTTAGTTTTTTAATCATCCTGTGAGGAACTCGGGGATTTAAATTTTCCGCT TCTTGTGGTGTTTGT**AG**ATGAATATTCCAGTATTCTGCCCAGGGTTAACAGATGGCTCTCTTGGGGATATG

IPVFCP G CTGTATTTTCACTCTTTTCGTACCTCTGGCCTCATCATCGATGTAGTACAAGGTACTTCTTTTACTCAATA YFHSFRTSGLI IDVV

AGTCAGTGTGATAAATATTCCTGCTACATCTAGTGCAGGAATATTGTAACTAGTAGTGCATTGTAGCTTTT CCAATTCAGCAACGGACTTTACTGTAAGTTGATATCTAAAGGTTCAAACGGGAGCTAGGAGAATAGCATAG GGGCATTCTGATTTAGGTTTGGGGCACTGGGTTAAGAGTTAGAGAATAATAATCTTGTTAGTTGTTTATCA AACTCTTTGATGGTTAGTCTCTTGGTAATTTGAATTTTATCACAGTGTTTATGGTCTTTGAACCAGTTAAT GTTTTATGAACAGATATCAGAGCTATGAACGGCGAAGCTGTCCATGCAAATCCTAAAAAGACAGGGATGAT

DIRAMNGEAVHANPKKTGM AATCCTTGGAGGGGGCTTGCCAAAGCACCACATATGTAATGCCAATATGATGCGCAATGGTGCAGATTACG I L G G G L P K H H I C N A N M M R N G A D Y CTGTATTTATAACACCGGGCAAGAATTTGATGGGAGCGACTCGGGTGCACGCCCTGATGAAGCCGTGTCT TGQEF D G S D S G A R TGGGGTAAAATTAGGGGTTCTGCTAAAACCGTTAAGGTCTGCTTTTTAATTTCTTCACATCCTAATTTATA WGKIRGSAKTVKVCFL ISSHPNL TCTCACTCAGTGGTTTTGAGTACATATTTAATATTGGATCATTCTTGCAGGTATACTGTGATGCTACCATA

GCCTTCCCATTGTTGGTTGCAGAAACATTTGCCACAAAGAGAGACCAAACCTGTGAGTCTAAGACTTAAGA ACTGACTGGTCGTTTTTGGCCATGGATTCTTAAAGATCGTTGCTTTTTTGATTTTTACACTGGAGTGACCATAT AACACTCCACATTGATGTGGCTGTGACGCGAATTGTCTTCTTGCGAATTGTACTTTAGTTTCTCTCAACCT AAAATGATTTGCAGATTGTGTTTTCGTTTAAAACACAAGAGTCTTGTAGTCAATAATCCTTTGCCTTATAA AATTATTCAGTTCCAACAACACATTGTGATTCTGTGACAAGTCTCCCGTTGCCTATGTTCACTTCTCTGCG

### Figure 2B

MEDDRVFSSVHSTVFKESESLEGKCDKIEGYDFNQGVDYPKLMRSMLTTGFQASNLGEAIDVVNQMFEFVLKLDWRLADETTV AEDCSEEEKNPSFRESVKCKIFLGFTSNLVSSGVRDTIRYLVQHHMVDVIVTTTGGVEEDLIKCLAPTFKGDFSLPGAYLRSK GLNRIGNLLVPNDNYCKFEDWIIPIFDEMLKEQKEENVLWTPSKLLARLGKEINNESSYLYWAYKMNIPVFCPGLTDGSLGDM LYFHSFRTSGLIIDVVQDIRAMNGEAVHANPKKTGMIILGGGLPKHHICNANMMRNGADYAVFINTGQEFDGSDSGARPDEAV SWGKIRGSAKTVKVCFLISSHPNLYLTQWF

### Figure 2C

GGTGGTGTTGAGGAAGATCTCATAAAATGCCTTGCACCTACATTTAAAGGTGATTTCTCTCTACCTGGAGCTTATTTAAG
GTCAAAGGGATTGAACCGAATTGGGAATTTGCTGGTTCCTAATGATAACTACTGCAAGTTTGAGGATTGGATCATTCCCA
TCTTTGACGAGATGTTGAAGGAACAGAAAGAAGAAGATGTGTTGTGGACTCCTTCTAAACTGTTAGCACGGCTGGGAAAA
GAAATCAACAATGAGAGTTCATACCTTTATTGGGCATACAAGATGAATATTCCAGTATTCTGCCCAGGGTTAACAGATGG
CTCTCTTAGGGATATGCTGTATTTTCACTCTTTTCGTACCTCTGGCCTCATCATCGATGTAGTACAAGATATCAGAGCTA
TGAACGGCGAAGCTGTCCATGCAAATCCTAAAAAGACAGGGATGATAATCCTTGGAGGGGGGCTTGCCAAAGCACCACATA
TGTAATGCCAATATGATGCGCAATGGTGCAGATTACGCTGTATTTATAAACACCCGGGCAAGAATTTGATGGGAGCGACTC
GGGTGCACGCCCTGATGAAGC

### Figure 2D

GGVEEDLIKCLAPTFKGDFSLPGAYLRSKGLNRIGNLLVPNDNYCKFEDWIIPIFDEMLKEQKEENVLWTPSKLLARLGKEIN NESSYLYWAYKMNIPVFCPGLTDGSLRDMLYFHSFRTSGLIIDVVQDIRAMNGEAVHANPKKTGMIILGGGLPKHHICNANMM RNGADYAVFINTGQEFDGSDSGARPDE

Figure 3

# Human, Arabidopsis, Tomato, Yeast, Neurospora(Fungi), and Multiple DHS Sequence Alignments of Methanococcus(Archaeobacteria)

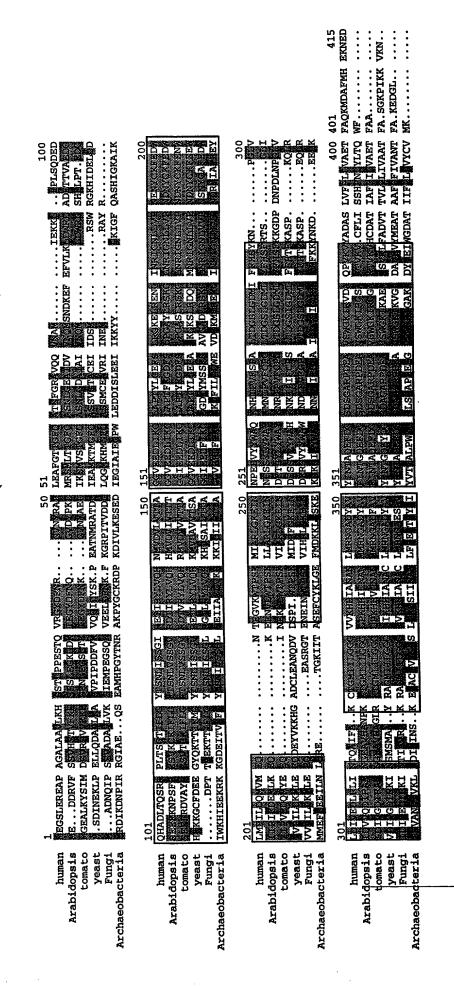
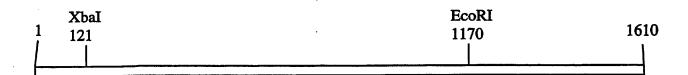
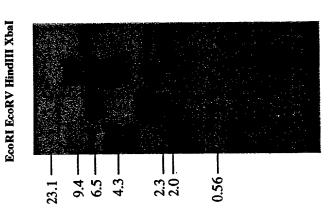


Figure 4



Southern Analysis of DHS



# Northern Analysis of DHS on **Tomato Flowers**

Blossom

Bud

and Senescence



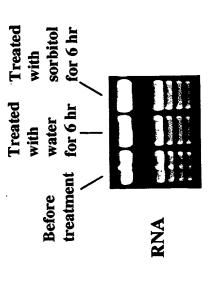
Northern

### Northern Analysis of DHS on Developmental Stages of Tomato Fruit

Ripe Breaker Pink (red)

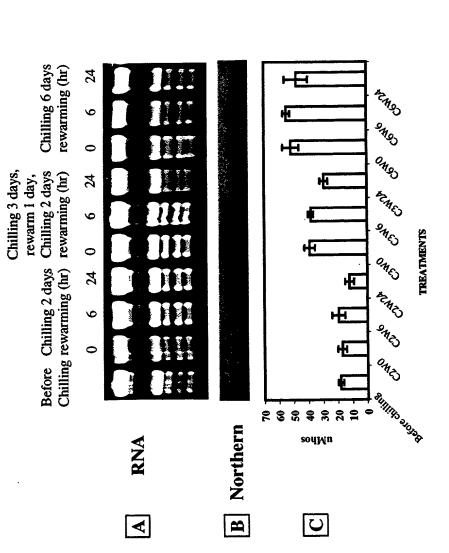
Northern Blot

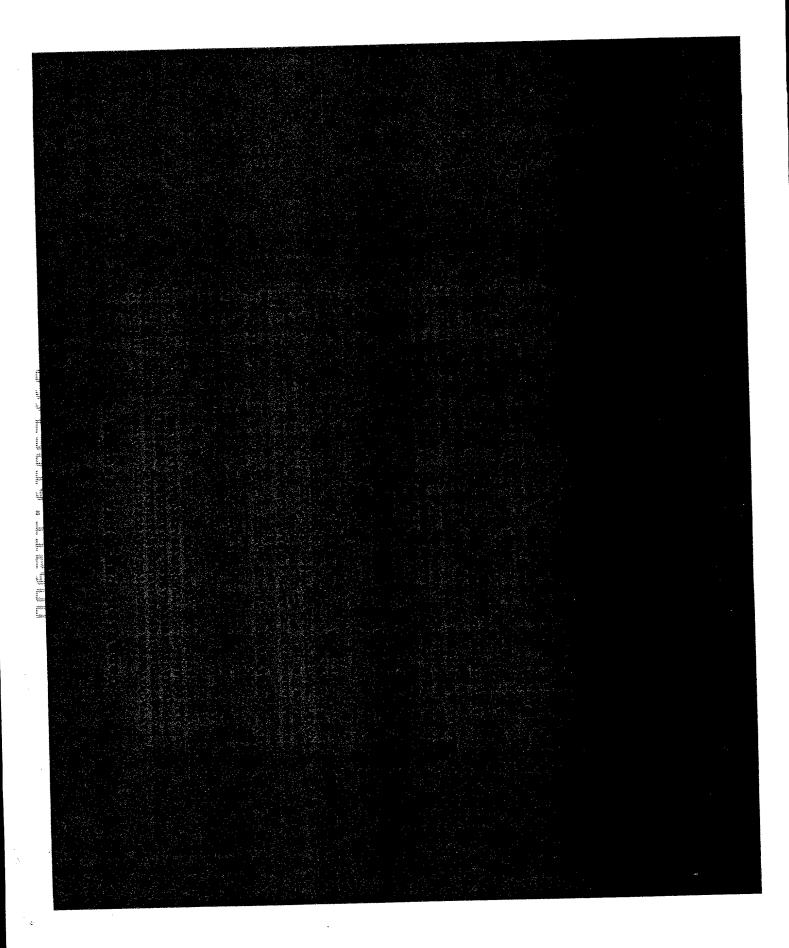
# Northern Analysis of DHS - 2M Sorbitol treated Tomato Leaves



Northern

# Northern Analysis of DHS Tomato Leaf Chilling Effects





Northern Analysis of WT AT Aging Leaves

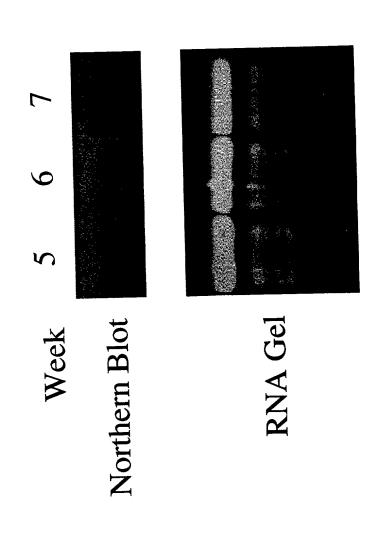


Figure 11

Northern Analysis of Canation Petal (In Situ)DHS

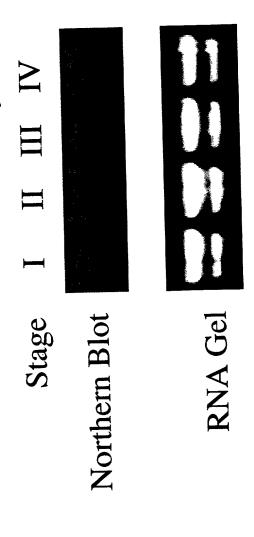


Figure 12

## Tomato eif5A

### Figure 13

GAAGAGCAGATTAACGCCGTTAAGGATGTTGGTACCAAGAAT**TAG**TTATGTCATGGCAGC GAAAGTGGAAACACCAAGGATGACCTCAGGCTTCCCACCGATGAAAATCTGCTGAAGCAG GTTAAAGATGGGTTCCAGGAAGGAAAGGATCTTGTGGTGTCTGTTATGTCTGCGATGGGC ACTTCAAAAACTGGAAAACACGGACATGCTAAATGTCACTTTGTGGCAATTGACATTTTT AATGGAAAGAAACTGGAAGATATCGTTCCGTCCTCCCACAATTGTGATGTGCCACATGTT AACCGTACCGACTATCAGCTGATTGATATCTCTGAAGATGGTTTTGTCTCACTTCTTACT CATTTTGAGTCAAAGGCAGATGCTGGTGCCTCAAAAACTTTCCCACAGCAAGCTGGAACC ATCCGTAAGAATGGTTACATCGTTATCAAAGGCCGTCCCTGCAAGGTTGTTGAGGTCTCC ტ **>** 回 Н Н 덜 Ø ρι 闰 K V V OI Н н ល Ω æ O) A Z ល > Д ບ Ē 闰 ပ Ī×ι Z ŋ Ē മ Ħ S H Д Ω H H **4** ပ 闰 × Д O ĸ ß ß н Н ტ Ø H K L E D I V P ĸ ¥ TDYQLIDI Ω > Ω 4 KDDL н (J) Ü O > Н Ħ 闰 Q I N A V Α × Oi × U O H [4 (U z U H Z ĸ V K D ល ŋ 闰

764 bps, not including Poly(A) tail; 160 amino acids

ATAATCACTGCCAAAGCTTTAAGACATTATCATATCCTAATGTGGTACTTTGATATCACT

CTAGAGAAAGTATTGGCTTTTGACAGCACAGTTGAACTATGTGAAAATTCTAC 

### Carnation - F5A

GGGAGAAGAGCAGATCTGCGCCGTCAAGGACGTTAGTGGTGGCAAG<u>TAG</u>A AGGATGATCTGAAGCTTCCTGCTGATGAGGCCCTTGTGAAGCAGATGAAG CACAATTGTGATGTTCCACATGTCAACCGTGTCGACTACCAGCTGCTTGA CCATTGACATTTTCAACGGCAAGAAGCTGGAAGATATTGTCCCCTCATCC TATCACTGAAGATGGCTTTGTTAGTCTGCTGACTGACAGTGGTGACACCA CTCTTTTACATCAATCGAAAAAAATTAGGGTTCTTATTTAGAGTGAGA GGCGAAAAATCGAACG<u>ATG</u>TCGGACGACGATCACCATTTCGAGTCATCGG CCGACGCCGGAGCATCCAAGACTTACCCTCAACAAGCTGGTACAATCCGC AAGAGCGGTCACATCATCAAAAATCGcCCtTGCAAGGtGGTTGAGGT TTCTACCTCCAAGACTGGCAAGCACGGTCATGCCAAATGTCACTTTGTTG HNCDVPHVNRVDYQLLD STSKTGKHGHAKCHFVA K S G H I V I K N R P C K V V E V D D L K L P A D E A L V K Q M K SKTYPQQAGTIR EGFEAGKDLILSVMCA SDDDHHFES G E E Q I C A V K D V S G G K IDIFNGKKEBDIVP

790 bps, 160 amino acids

TCGAGAACATTCTGAACCTTATATGTTGAATTGATGGTGCTTAGTTTGTT TTGGAAATCTCTTTGCAATTAAGTTGTACCAAATCAATGGATGTAATGTC TTGAATTTGTTTTATTTTTGTTTTGATGTTTTGCTGtGATTGCATTATGCA

AGCTTTTGATGAATCCAATACTACGCGGTGCAGTTGAAGCAATAGTAATC

# Arabi dopsis F5A

CTGTTACCAAAAATCTGTACCGCAAAATCCTCGTCGAAGCTCGCTGCTGCAACCAAGTC

TGAGGTTTCAACCTCGAAGATGCATGGTCATGTAAATGTCATTTTGTAGCTAT EVSTREVAR TCCTCATGTCAACCGTACTGATTATCAGCTGATTGACATTTCTGAAGATGGATATGTCAG TTTGTTGACTGATAACGGTAGTACCAAGGATGACCTTAAAGCTCCCTAATGATGACACTCT GCTCCAACAGATCAAGAGT**GGGTTTGATGAAAGAAAGA**TCTAGTGGTGAGTGTAATGTC AGCTATGGGAGAGGAACAGATCAATGCTCTTAAGGACATCGGTCCCAAG**TGA**GACTAACA CGACGAGGAGCATCACTTTGAGTCCAGTGACGCCGGAGCGTCCAAAAACCTACCCTCAACA AGCTGGAACCATCCGTAAGAATGGTTACATCGTCATC**AAAAATCGTCCCTGCAAGGT**TGT LLTDNGSTKDDLKLPNDDTL A G T I R K N G Y I V I K N R P C K V V PHVNRTDYQLIDISEDGYV LQQIKSGFDDGKDLVVSVM H F E S S D A G A S K T Y P A M G E E Q I N A L K D I G P K EVSTSKTGKHGHAK DIFTSKKLEDIVPS

**AAGCCTCCCCTTTGTTATGAGATTCTTCTTCTTGTAGGCTTCCATTACTCGTCGGAGA** TTATCTTGTTTTTGGGTTACTCCTATTTTGGATATTTTAAACTTTTTGTTAATAATGCCATC TTCTTCAACCTTTTCCTTTCTAGATGGTTTTTTATACTTCTTCT

754 bps, not including Poly(A) tail; 158 amino acids

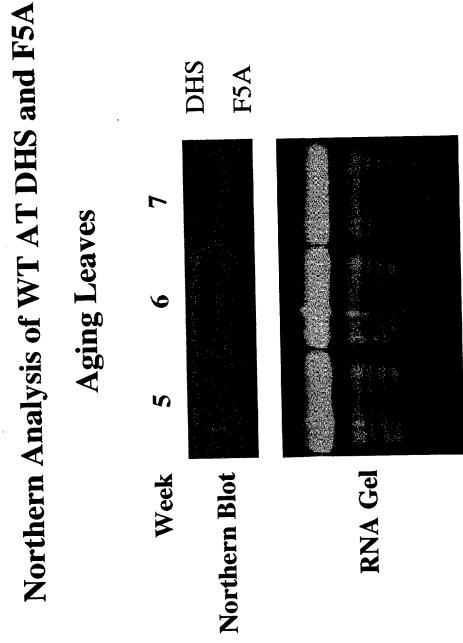


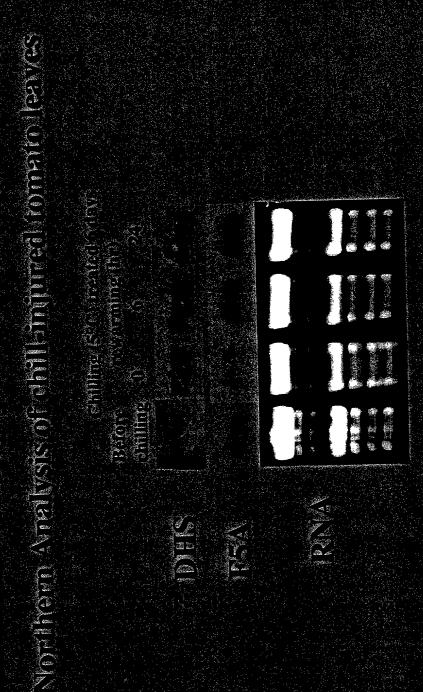
Figure 16

Figure 17

Figure 18



Figure 19



### Wild-Type



3.1 Weeks

Figure 21

# 4.6 Weeks

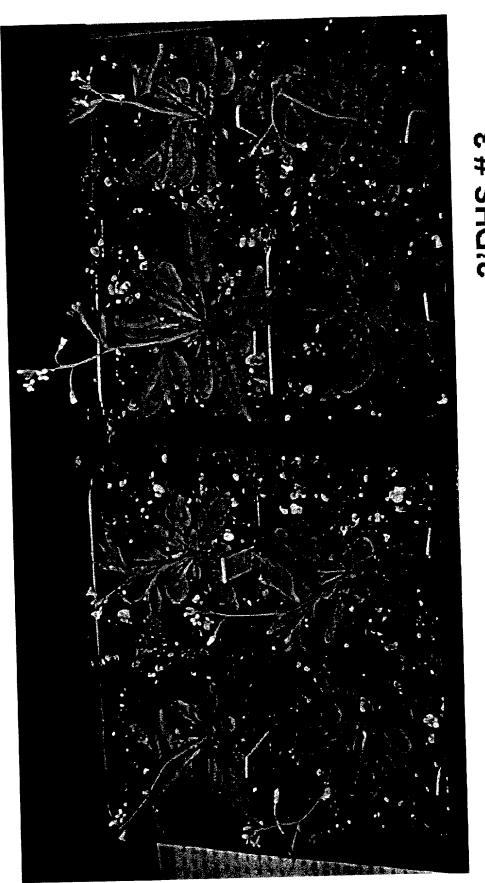
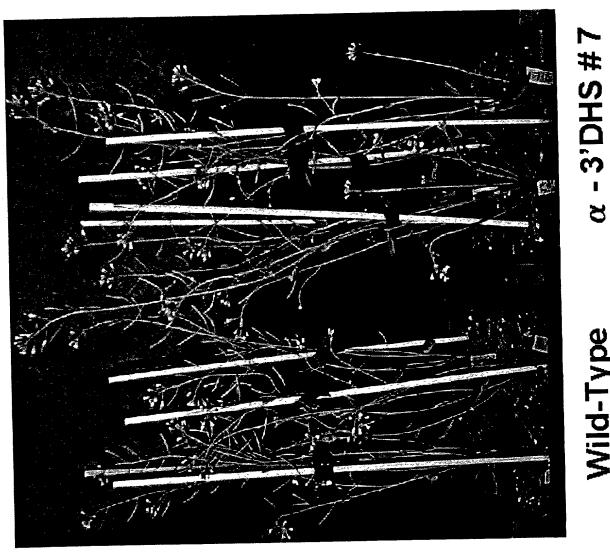


Figure 22

Figure 23

# 6.1 Weeks



Seed Volume of Transgenic antisense-3'DHS plants anti-3'DHS # 5-5 anti-3'DHS # 8-2 Seed Volume anti-3'DHS # 1-1 AVG. WT 0 200 1400 <sub>1</sub> 400 800 009 1200 1000

Plant Line

Figure 25

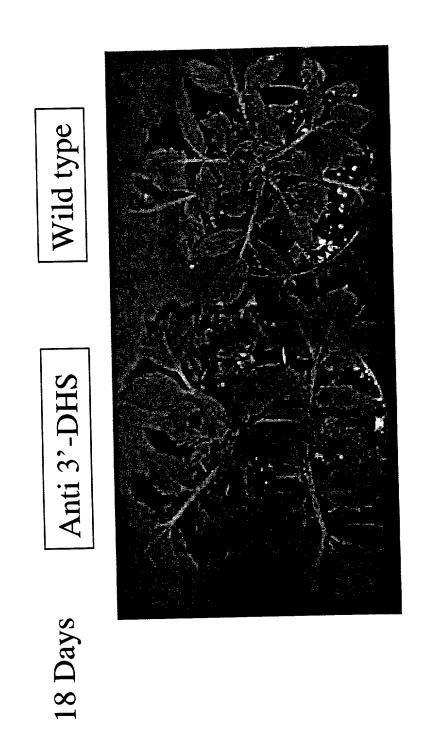


Figure 26

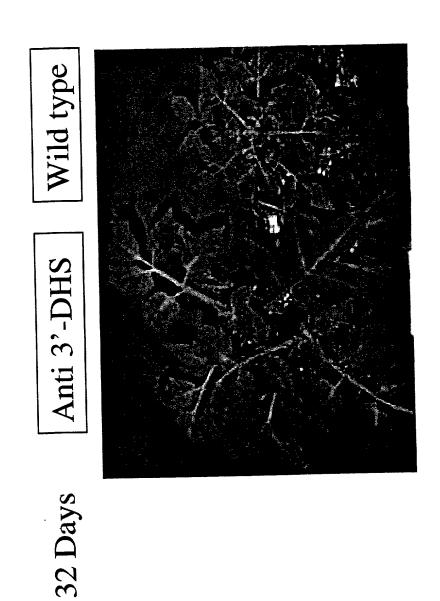


Figure 27

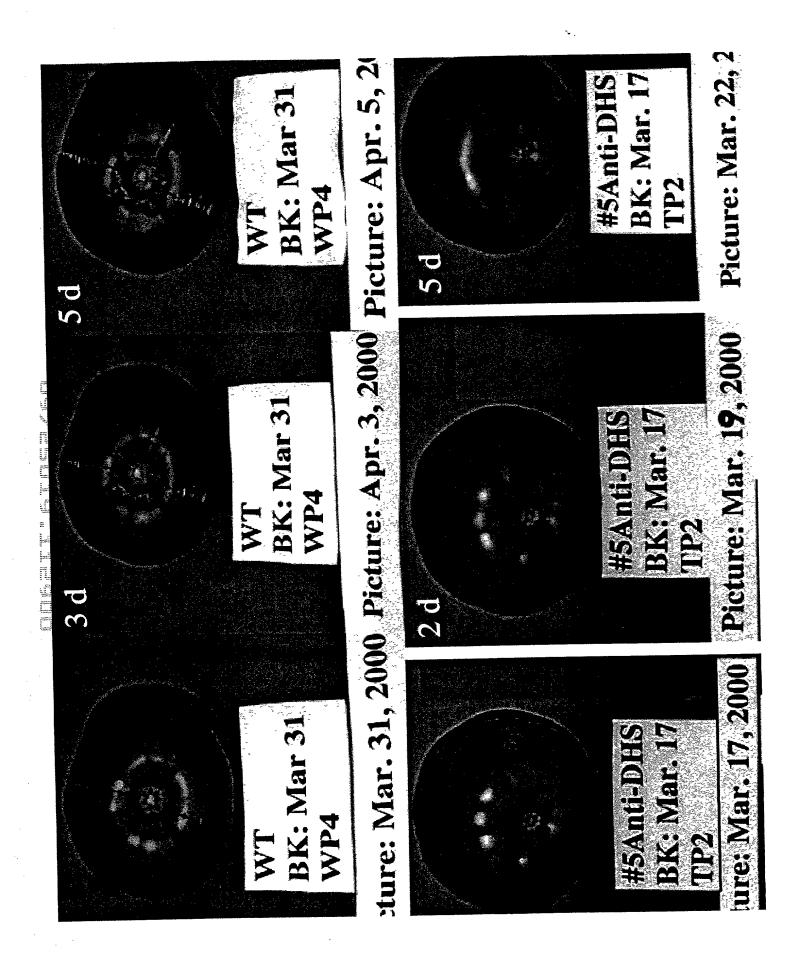


Figure 28

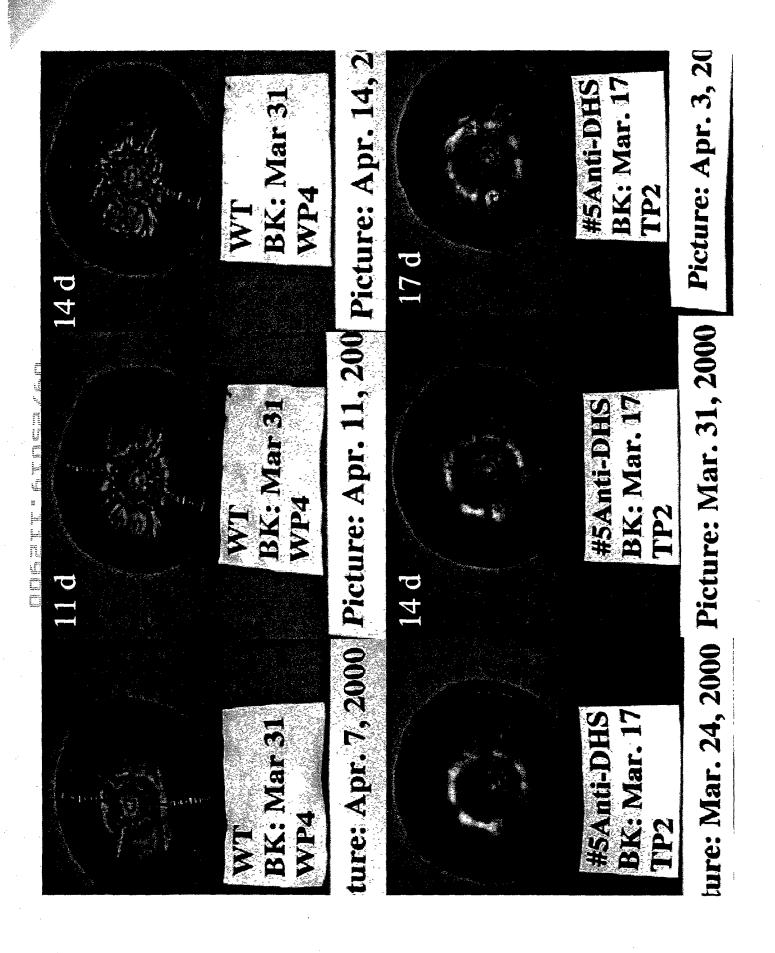


Figure 29

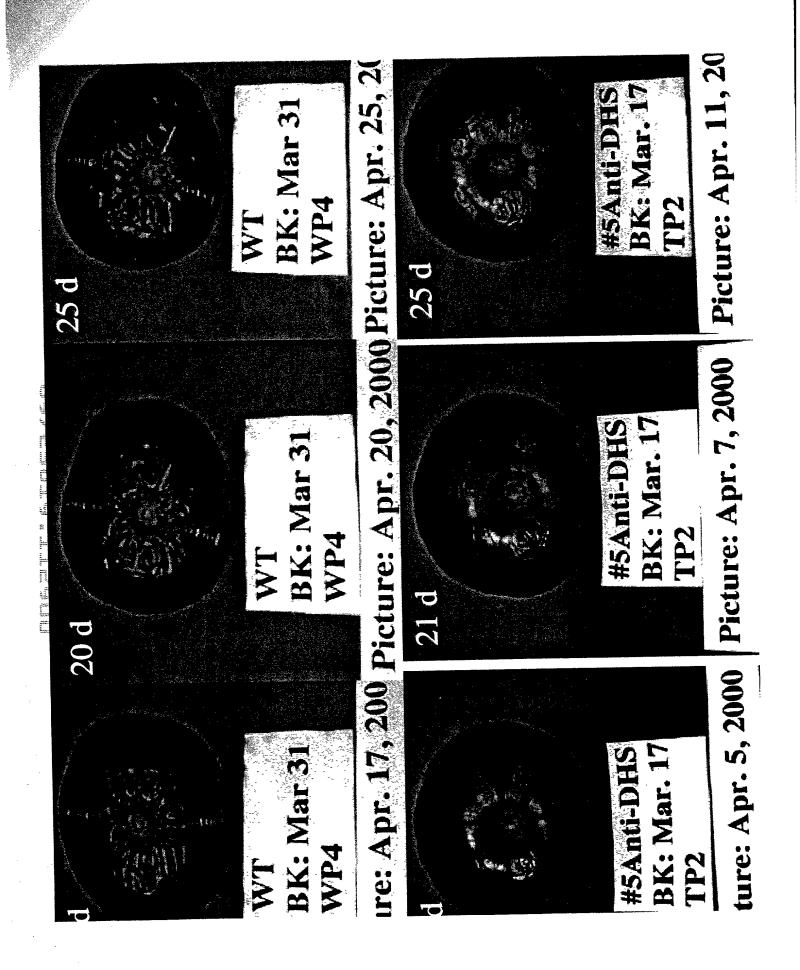


Figure 30

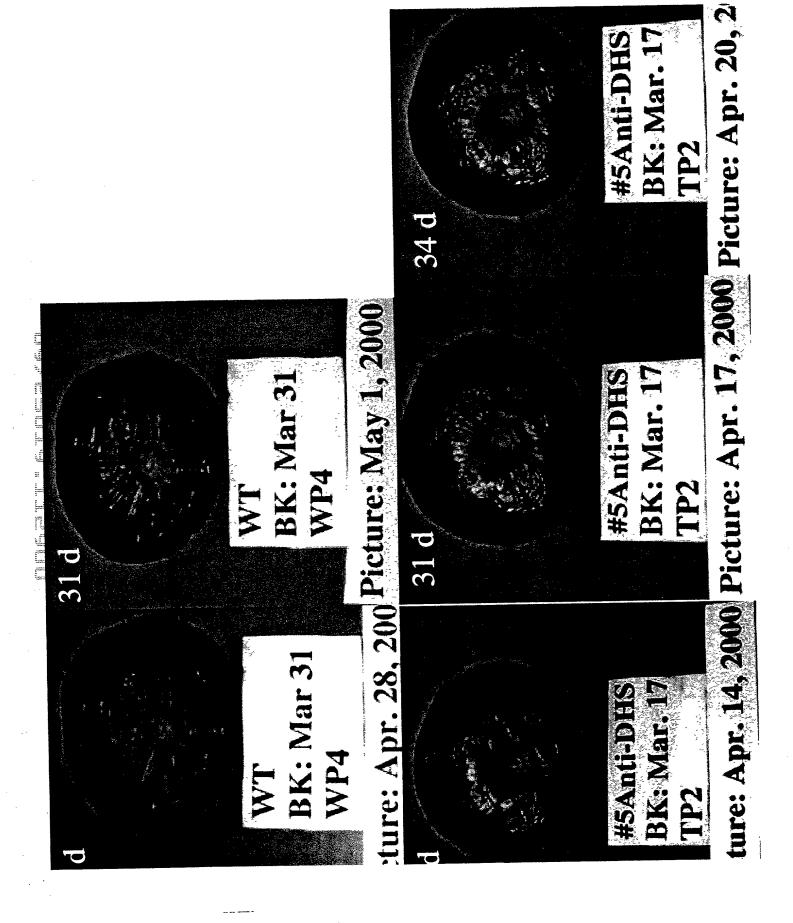


Figure 31

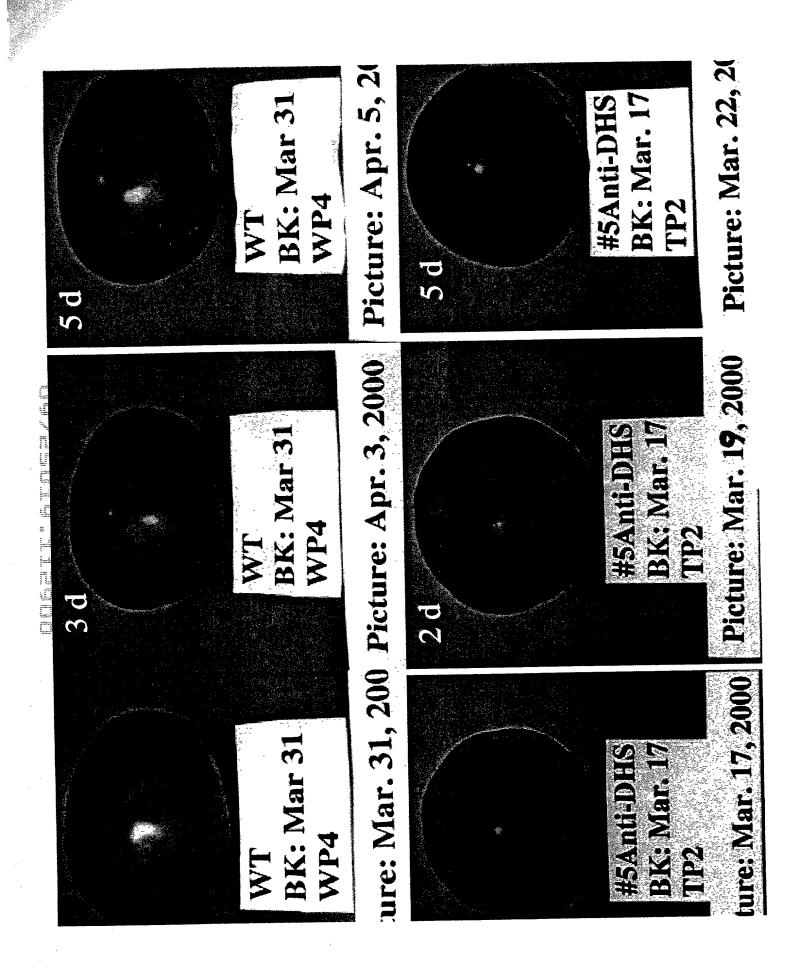


Figure 32

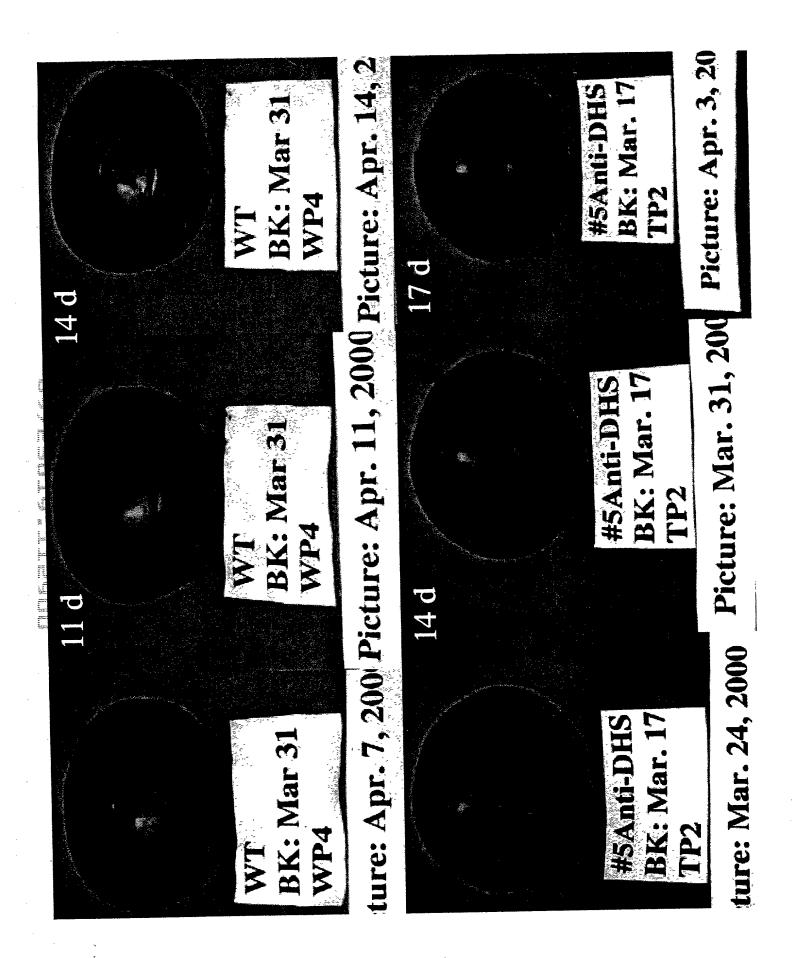


Figure 33

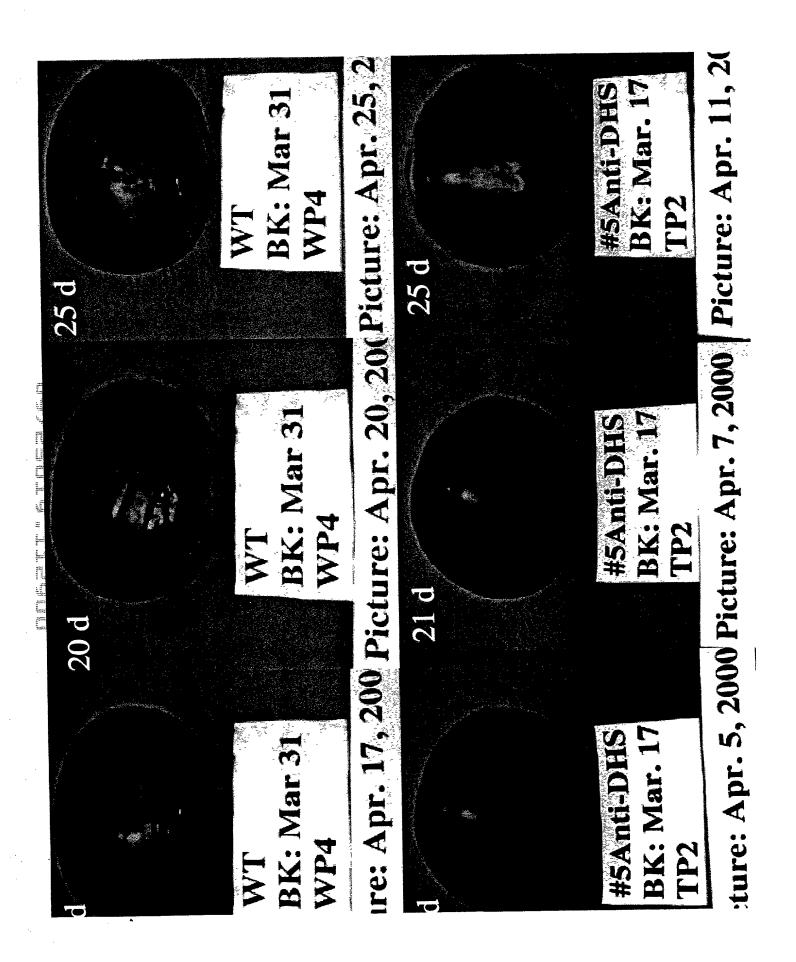


Figure 34

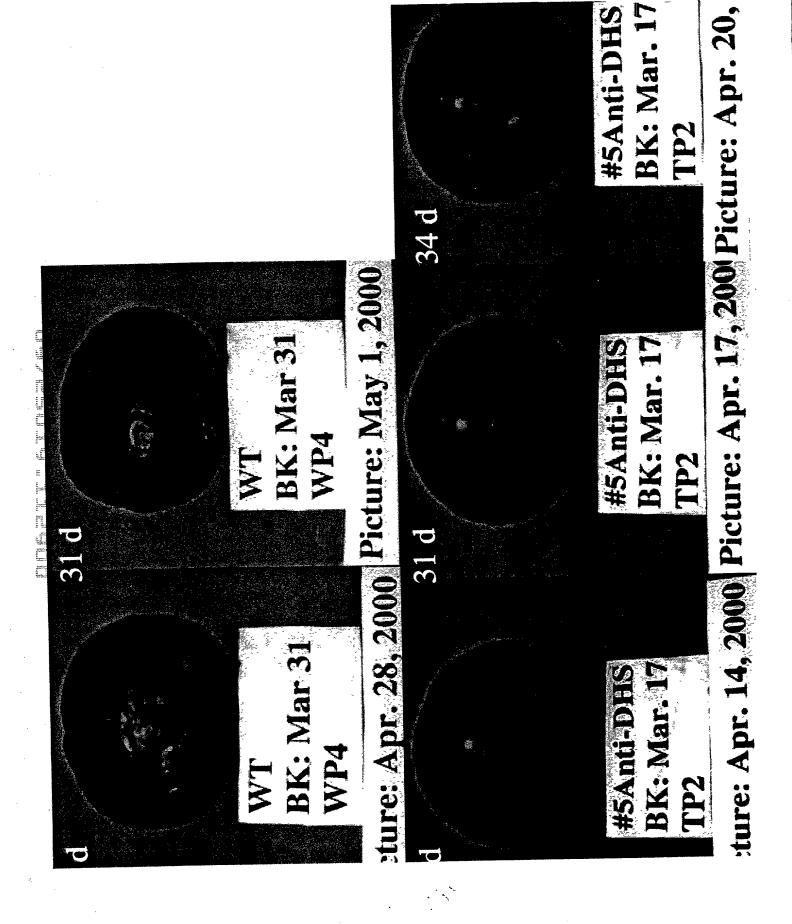


Figure 35

### Arabidopsis 3'-end DHS for antisense

Nucleotide and derived amino acid sequence
TGCACGCCTGATGAAGCTGTGTCTTGGGGTAAAATTAGGGGTTCTGCTAAAACCGTTAAGGTCTGCTTTT
ARPDEAVSWGKIRGSAKTVKVCF

TAATTTCTTCACATCCTAATTTATATCTCACTCAGTGGTTTTGAGTACATATTTAATATTGGATCATTCTT L I S S H P N L Y L T Q W F

Nucleotide sequence

ARPDEAVSWGKIRGSAKTVKVCFLISSHPNLYLTQWF

### Tomato 3'-end-Deoxyhupsine synthase used for antisense

Nucleotide and derived amino acid sequence
GGTGCTCGTCCTGATGAAGCTGTATCATGGGGAAAGATACGTGGTGCCAAGACTGTGAAGGTGCATTGTGATGCAAC
G A R P D E A V S W G K I R G G A K T V K V H C D A T

CATTGCATTTCCCATATTAGTAGCTGAGACATTTGCAGCTAAGAGTAAGGAATTCTCCCAGATAAGGTGCCAAGTTTGAA
I A F P I L V A E T F A A K S K E F S Q I R C Q V

Nucleotide sequence GGTGCTCGTCCTGATGAAGCTGTATCATGGGGAAAGATACGTGGTGGTGCCAAGACTGTGAAGGTGCATTGTGATGCAAC CATTGCATTTCCCATATTAGTAGCTGAGACATTTGCAGCTAAGAGTAAGGAATTC

### 600 bp Arabidopsis Deoxyhypusine Synthase Probe

### Primer1 (underlined)

 ${\tt GGTGGTGTTGAGGAAGATC}{\tt TCATAAAATGCCTTGCACCTACATTTAAAGGTGATTTCTCTCTACCTGGAGC}$ TTATTTAAG A P Y L R GTCAAAGGGATTGAACCGAATTGGGAATTTGCTGGTTCCTAATGATAACTACTGCAAGTTTGAGGATTGGA TCATTCCCA V P D N TCTTTGACGAGATGTTGAAGGAACAGAAAGAAGAAGATGTGTTGTGGACTCCTTCTAAACTGTTAGCACGG **CTGGGAAAA** E N V K L G K GAAATCAACAATGAGAGTTCATACCTTTATTGGGCATACAAGATGAATATTCCAGTATTCTGCCCAGGGTT AACAGATGG A Y K M N T D G CTCTCTTAGGGATATGCTGTATTTTCACTCTTTTCGTACCTCTGGCCTCATCATCGATGTAGTACAAGATA **TCAGAGCTA** R Α TGAACGGCGAAGCTGTCCATGCAAATCCTAAAAAGACAGGGATGATAATCCTTGGAGGGGGCTTGCCAAAG CACCACATA K T G M H H I TGTAATGCCAATATGATGCGCAATGGTGCAGATTACGCTGTATTTATAAACACCGGGCAAGAATTTGATGG GAGCGACTC DYAV Ι R N G A S D S GGGTGCACGCCCTGATGAAGC GARPDE

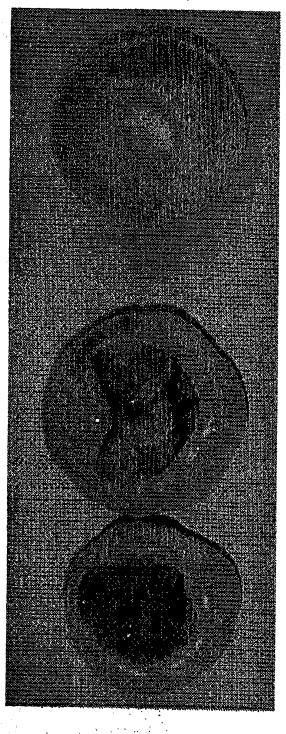
Primer 2 (underlined)

### 483 bp Carnation Deoxyhypusine Synthase Probe

GAAGATCCATCAAGTGCCTTGCACCCACTTTCAAAGGCGATTTTGCCTTACCAGGAGCTCAATTACGCTCC TGAATCGAATTGGTAATCTGTTGGTTCCGAATGATAACTACTGTAAATTTGAGGATTGGATCATTCCAATT **TTAGATA** NDNYC P K AGATGTTGGAAGAGCAAATTTCAGAGAAAATCTTATGGACACCATCGAAGTTGATTGGTCGATTAGGAAGA EKIL ACGATGAGAGTTCATACCTTTACTGGGCCTTCAAGAACAATATTCCAGTATTTTGCCCAGGTTTAACAGAC K TCGGAGACATGCTATATTTTCATTCTTTTCGCAATCCGGGTTTAATCATCGATGTTGTGCAAGATATAAGA **GCAGTAA** ATGGCGAGGCTGTGCACGCAGCGCCTAGGAAAACAGGCATGATTATACTCGGTGGAGGGTTGCCTAAGCAC Α GCAACGCAAACATGATGAGAAATGGCGCCGATTATGCTGTTTTCATCAACACCG CNANMMRNGADYAVFINT

A full-length cDNA clone was obtained by screening a carnation senescing petal cDNA library with this probe.

Figure 40A



Blossom end rot

Normal

